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Research article
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Determination of the ciprofloxacin-resistant *Escherichia coli* isolated from chicken meat in Turkey

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ABSTRACT. In this study, the occurrence of the ciprofloxacin-resistant (CR) *Escherichia coli* in chicken meat was determined, and their clonal relations were investigated by using pulsed-field gel electrophoresis (PFGE). Antimicrobial resistance patterns of *E. coli* isolates were determined by using disc diffusion assay, and minimum inhibitory concentration of ciprofloxacin was determined by E-test. Plasmid-mediated quinolone resistance (PMQR) and extended spectrum beta-lactamase (ESBL) resistance genes were also screened through polymerase chain reactions. Sixty chicken meat samples were collected from different supermarkets and butchers in Sivas, Turkey. CR *E. coli* strains were determined in 59 (98.3%) chicken meat samples. By analyzing PFGE fingerprint data, 34 different pulsotypes were determined. All *E. coli* strains were found to be resistant to nalidixic acid, enrofloxacin, and norfloxacin. In addition, isolates were resistant to levofloxacin (40.7%), ampicillin (94.9%), trimethoprim-sulfamethoxazole (76.3%), tetracycline (69.5%), and chloramphenicol (44.1%). However, isolates were susceptible to imipenem and colistin. In this study, 81.4% of CR *E. coli* isolates were observed to have a multidrug-resistant profile, which is defined as resistance to three or more classes of antibiotics. Through phenotypic confirmation tests, five isolates (8.3%) were determined to be ESBL-producing. The PMQR genes were not determined in any of the isolates. Two isolates (3.4%) possessed the $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ genes, and 40 isolates (67.8%) had the bla_{TEM} gene. Taken together, retail raw chicken meat is highly contaminated with CR *E. coli*. However, these isolates are not found to be carriers of the PMQR genes, indicating a low public health problem.

Keywords: Ciprofloxacin resistance; chicken meat; *E. coli*; plasmid-mediated quinolone resistance

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INTRODUCTION

Escherichia coli are commonly found in the gastrointestinal tract of humans and warm-blooded animals and can cause enteritis, urinary tract infection, septicemia, pneumonia, and meningitis in humans and animals (Allocati et al., 2013; Ray, 2004). The emergence of antimicrobial resistance threatens the treatment of *E. coli* infections. The higher prevalence of multidrug-resistant (MDR) *E. coli* strains have increased worldwide in the past few decades (Allocati et al., 2013; EFSA, 2019; Mavroidi et al., 2012). It is a well-known fact that the primary reason for the high prevalence of MDR *E. coli* is the unregulated use of antimicrobial agents in humans and animals (Seo and Lee, 2019).

Quinolones are broad-spectrum antibiotics widely used against gram-negative bacteria, including *E. coli* infections in human and veterinary medicines, which has eventually resulted in the rapid emergence of quinolone resistant bacteria (Borjesson et al., 2016; EFSA, 2019; WHO, 2007). The first generation of quinolone molecules were licensed for use in food animals at the beginning of the 1980s, and fluoroquinolones were licensed during the late 1980s and early 1990s (EMEA, 2006). Since then, new fluoroquinolone molecules have been authorized, and a number of different veterinary medicines are now available on the market (EMEA, 2006; Gouvea et al., 2015).

Two main mechanisms of quinolone resistance have been described: (i) alterations in the targets of quinolones, and (ii) decreased accumulation inside the bacteria due to decreased permeability of the membrane and/or an overexpression of efflux pump systems. Both of these mechanisms are noted to be chromosomally mediated (Ruiz, 2003). Chromosomal mutations located in the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *gyrB* genes, which encode for the two DNA gyrase (topoisomerase II) subunits, and the *ParC* and *ParE* genes, which encode for two topoisomerase IV subunits (Jacoby, 2005). In addition, plasmid-mediated quinolone resistance (PMQR) genes were also noted to contribute to quinolone resistance by either altering the molecular structure of quinolone target enzymes or the enzymatic inhibition of quinolones (Martinez-Martinez et al., 1998).

PMQR genes, first reported in a pMG252 plasmid from a *Klebsiella pneumoniae* strain, were obtained from a patient's urine specimen at the University of Alabama, Birmingham in 1994 (Martinez-Martinez et al., 1998). To date, different PMQR determinants have been identified, the *qnr* families, *aac(6')-Ib-cr* (drug modification), and *qepA* and *oqxAB* (active efflux pumps) (Rodriguez-Martinez et al., 2016), and are usually associated with mobile elements on plasmids often found to be incorporated into *sul1*-type integrons. Their sequences have been uploaded into the following web-based database: http://www.lahey.org/qnrStudies/ (Jacoby et al., 2014; Martinez-Martinez et al., 2008). Importantly, mobile genetic elements carrying PMQR genes commonly carry other important antimicrobial resistance traits, such as extended spectrum β-lactamase (ESBL) genes (Robicsek et al., 2006; Allocati et al., 2013).

Despite the fact that the quinolones have never been used in Norway livestock production, ciprofloxacin resistance was determined by using selective method. These particular situations paved the way for investigations of PMQR genes in food from animal origins, especially in chicken meat, and were found to be significant from a public health point of view (Slettemeas et al., 2019). Although ciprofloxacin has been widely used in human and veterinary medicines in Turkey (Nazik et al., 2008; Şahintürk et al., 2016). Thus, the main aim of this study was to determine the occurrence of ciprofloxacin resistant *E. coli* strains by using selective enrichment and molecular characterization of PMQR (*qnrA, qnrB, qnrS, qnrC* and *aac(6')-lb-cr*) in these isolates. The isolates were further characterized by pulsed-field gel electrophoresis (PFGE), and the presence of genes encoding different β-lactamases ($bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, bla_{TEM} and bla_{SHV}) was investigated.

MATERIALS AND METHODS

Sample Collection and Isolation of Ciprofloxacin Resistant *E. coli*

Sixty chicken meat samples were collected from different supermarkets and butchers from September to December 2018 in Sivas, Turkey. For the *E. coli* isolation, each chicken meat sample (25 g) was suspended in 225 mL of sterile Buffered peptone water (Oxoid CM0509) and was mixed by paddle blender (Interscience Bag Mixer, France) for 2 min. Then, the homogenate was incubated for 24 h at 37°C. At the end of the incubation, 100 μL of the enrichment was plated on a Tryptone bile x-glucuronide (TBX) agar plate (Oxoid, CM0945), including 0.5 μg/mL ciprofloxacin (Sigma-Aldrich, U.S.A), and incubated for 3 h at 44°C and then 24 h at 41°C. After incuba-

tion, green *E. coli* colonies (one colony per sample) isolated from each sample were subcultured on Columbia blood agar (Oxoid, CM0331). Isolates were stored in Tryptone soya broth (Oxoid CM0129) with 20% glycerol at -20°C. Species identification was performed using a MALDI-TOF (Bruker Daltonik GmbH, Leipzig, Germany). To identify bacterial species, each peak was directly matched against reference libraries and a result was considered valid (accurate identification to the species level) if the score value was ≥ 2.0. When the scores obtained were < 2.0, the samples were reevaluated.

Pulsed-Field Gel Electrophoresis

The genetic relatedness of the *E. coli* isolates in the current study was assessed by a PFGE procedure, and band profile analyses were performed by the Public Health Institution of Turkey (Ankara), as described previously (Durmaz et al., 2009), with XbaI restriction of DNA. The DNA band profiles were analyzed by using the BioNumerics software system (Applied Maths, Sinth-Martens-Latem, Belgium). A 1% band tolerance was used for the comparison of DNA profiles. Cluster analysis was done by the unweighted pair group method using with arithmetic mean (UPGMA). The level of the Dice similarity between patterns was defined at ≥ 85%.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibilities of all the CR *E. coli* isolates were determined by the disc diffusion method in accordance with the CLSI guidelines (CLSI, 2015). The following discs of antibiotics (Bioanalyse, Turkey) were used: amoxicillin-clavulanic acid (AMC; 20/10 μg), ampicillin (AM; 10 μg), aztreonam (ATM; 30 μg), cefoxitin (FOX; 30 μg), cefuroxime (CXM; 30 μg), cefpodoxime (CPD; 30 μg), colistin (CT; 10 μg), imipenem (IPM; 10 μg), chloramphenicol (C; 30 μg), gentamicin (CN; 10 μg), tetracycline (TE; 30 μg), nalidixic acid (NA; 30 μg), ciprofloxacin (CIP; 5 μg), enrofloxacin (ENR; 30 μg), levofloxacin (LEV; 5 μg), norfloxacin (NOR; 30 μg), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 μg), and bacitracin (B; 30 μg). *E. coli* (ATCC 25922) was used as the standard strain in the disc diffusion assay, and the results were interpreted according to CLSI guidelines (CLSI, 2015). In addition, E-tests (Bioanalyse, Ankara, Turkey) determined the minimum inhibitory concentrations (MICs) of ciprofloxacin. Briefly, broth suspensions of *E. coli* strains equivalent to a 0.5 McFarland standard were prepared and inoculated on Mueller-Hinton agar (Oxoid CM0337) plate. E-test strips were placed on the dry medium, which was then incubated under aerobic conditions for 24 h at 37°C. The cutoff breakpoints for ciprofloxacin were set at ≥ 4 μg/mL in accordance with the CLSI's epidemiological cutoff values (CLSI, 2015).

Determination and Characterization of PMQR and β-Lactamase Genes

All primers in this study are listed in Table 1. The genomic DNA was extracted by a standard boiling method, and the presence of the PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD* and *aac(6')-Ib-cr*) were determined by PCR (Park et al., 2006; Robicsek et al., 2006). PCR reactions were prepared as 50 μL [5 μL 10x PCR buffer, 5 μL 25 mM $MgCl_2$, 250 μM from each dNTP, 1.25 U Taq DNA Polymerase (MBI, Fermentas), 50 ρmol for each primer, and 25 ηg genomic DNA]. The PCR protocol was conducted by using a Bio-Rad T100 gradient thermal cycler device (BioRad, California, USA). The PCR conditions were carried out in the following steps: initial denaturation at 94°C for 45 s (denaturation at 53°C for 45 s and hybridization at 72°C for 60 s) for 32 cycles. Screening for *aac(6')-Ib-cr* was carried out by PCR amplification, as previously described by Park et al. (2006) PCR conditions were carried out through the following steps: initial denaturation at 94°C for 45 s (denaturation at 55°C for 45 s and hybridization at 72°C for 45 s) for 34 cycles. PCR products were run on electrophoresis in 1.5% (w/v) agarose gel and stained with ethidium bromide (10 mg/mL) for 30 min, and then screened under an ultraviolet transilluminator (Vilber Lourmat Quantum ST4, Marne-la-Vallee Cedex 1, France), using a 100 bp DNA ladder (MBI, Fermentas) as reference. To detect CTX-M, CMY-2, TEM, and SHV type β-lactamase, the $bla_{\text{CTX-M,}}$ $bla_{\text{CMY-2,}}$ bla_{TEM}, and bla_{SHV} genes were also amplified by PCR assay, which was conducted as described (Hasman et al., 2005; Leinberger et al., 2010; Mulvey et al., 2003). The β-lactamase genes were confirmed by sequencing the PCR products, as described previously (Ahmed et al., 2007). PCR-positive amplicons were obtained from National Food Institute EURL-AR reference strains collection, Technical University of Denmark were used, and distilled water was used for the negative control. (https://www.eurl-ar.eu/resources.aspx-#refstrains)

Table 1. Primer sequence of the used in PCR

Genes	Primer sequence (5´-3´)	Product size (bp)	References
*qnr*A	F:GGATGCCAGTTTCGAGGA R:TGCCAGGCACAGATCTTG	492	Cavaco et al., 2008
*qnr*B	F:GGMATHGAAATTCGCCACTG R:TTTGCYGYYCGCCAGTCGAA	262	Cattoir et al., 2007
*qnr*S	F:TCGACGTGCTAACTTGCG R:GATCTAAACCGTCGAGTTCGG	466	Cavaco et al., 2008
*qnr*C	F:GGGTTGTACATTTATTGAATCG R:CACCTACCCATTTATTTTCA	307	Jacoby et al., 2009
*qnr*D	F:CGAGATCAATTTACGGGGAATA R:AACAAGCTGAAGCGCCTG	582	Cavaco et al., 2009
aac(6')-Ib-cr	F:TTGCGATGCTCTATGAGTGGCTA R:CTCGAATGCCTGGCGTGTTT	482	Park et al., 2006
$bla_{\text{CTX-M}}$	F:ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYCAGCGG	593	Mulvey et al., 2003
$bla_{\text{CMY-2}}$	F:GCACTTAGCCACCTATACGGCAG R:GCTTTTCAAGAATGCGCCAGG	758	Hasman et al., 2005
bla_{TEM}	F:TGAGTATTCAACATTTCCGTGT R:TTACCAATGCTTAATCAGTGA	861	Leinberger et al., 2010
bla_{SHV}	F:CAAAACGCCGGGTTATTC R:TTAGCGTTGCCAGTGCT	937	Leinberger et al., 2010

RESULTS

In this study, 59 (98.3%) CR *E. coli* isolates were obtained from 60 chicken meat samples. Of these 59 isolates, 34 different pulsotypes were obtained when the similarity threshold was taken as ≥ 85%. Among these pulsotypes, 18 isolates were determined as clonally unrelated, and the remaining 41 were found to be related, which resulted in 16 groups with two to four isolates in each group (Fig 1).

The results of the antimicrobial susceptibility tests of the CR *E. coli* strains from chicken meat samples are shown in Table 2. Fifty-nine CR *E. coli* isolates and the susceptibility to 12 antimicrobial classes of these isolates were obtained from chicken meat samples. According to the results, all *E. coli* strains (100%) were determined as resistant to B, NA, ENR, and NOR. However, 40.7% of the strains were determined resistant to LEV, and 94.9%, 76.3%, 69.5%, and 44.1% of the *E. coli* strains were determined resistant to AM, SXT, TE, and C, respectively. On the other hand, all *E. coli* strains were susceptible to IPM and CT. Additionally, susceptibility to FOX and ATM were 93.2% and 96.6% among all *E. coli* strains, respectively. MDR (≥ 3 different antimicrobial classes) was observed in 48 (81.4%) of the 59 isolates (Table 3). MDR profiles were assessed as the following: seven (14.6%) isolates were resistant to three antimicrobial classes, 11 (22.9%) isolates were resistant to four, 15 (31.3%) isolates were resistant to five, nine (18.7%) isolates were resistant to six, five (10.4%) isolates were resistant to seven, and one (2.1%) isolate was resistant to eight antimicrobial classes. MIC values of CIP-resistant *E. coli* strains were determined as follows: ≥ 32 μg/mL (42 isolates), ≥ 16 μg/mL (three isolates), ≥ 12 μg/mL (two isolates), ≥ 8 μg/mL (seven isolates), and ≥ 6 μg/mL (three isolates; Fig 1).

None of the PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD,* and *aac(6')-Ib-cr*) were present among the isolates. Forty-six of the 59 CR *E. coli* isolates carried *β*-lactamase genes. Table 4 summarizes the results of the *β*-lactamase genes detected in CR *E. coli* isolates in this study. The bla_{TEM} gene was observed as predominant in CR *E. coli* isolates, and β-lactamase gene characterization revealed that 67.8% of the isolates had the bla_{TEM} gene (n = 40), 3.4% of the isolates had the $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ genes (n = 2), 3.4% of the isolates had the bla_{TEM} and $bla_{\text{CTX-M}}$ genes (n = 2), and 3.4% of the isolates had the bla_{TEM} and $bla_{\text{CMY-2}}$ genes (n = 2). None of the *E. coli* isolates contained the bla_{SHV} gene (Table 4). All the ampicillin-resistant isolates possessed the bla_{TEM} gene.

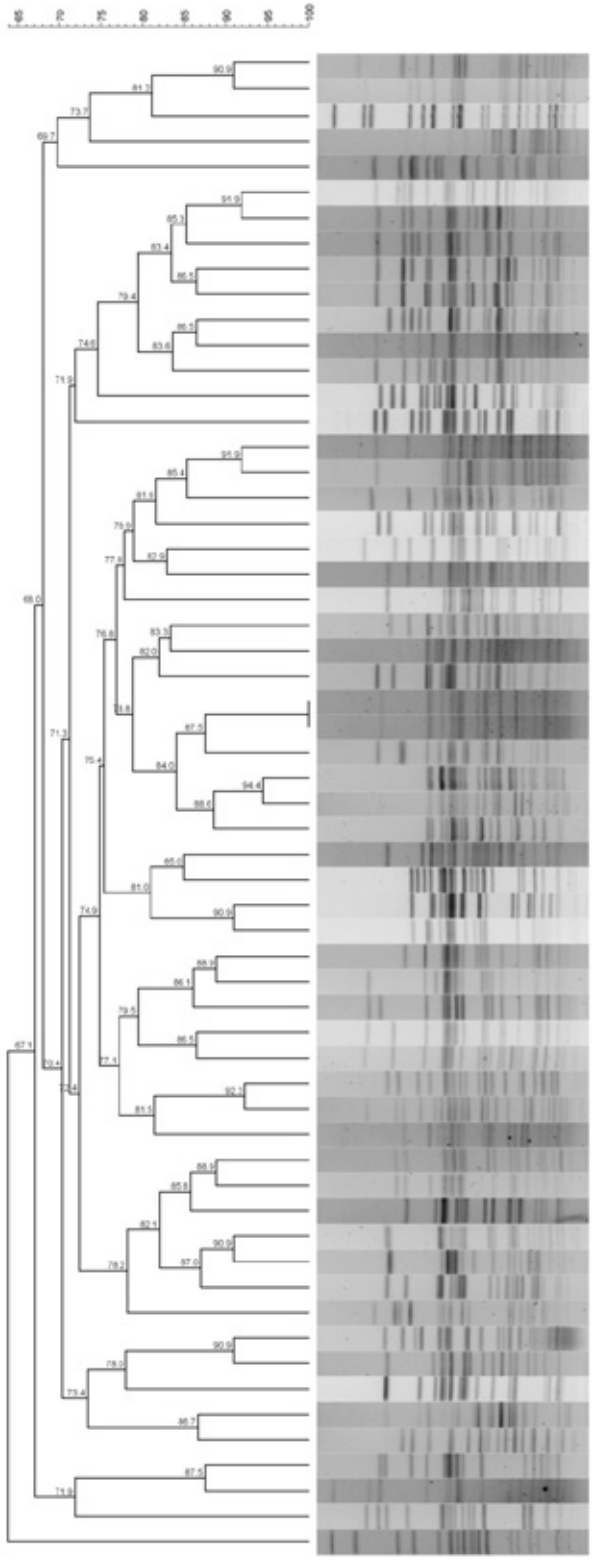
Table 2. Antimicrobial resistance of ciprofloxacin-resistant *Escherichia coli* isolated from chicken meat by disc diffusion assay (n = 59)

Antimicrobial Class	Antimicrobials	Number of isolates (%)		
		Resistant	Intermediate	Susceptible
Quinolones	Nalidixic acid	59 (100)	0	0
	Ciprofloxacin	56 (94.9)	3 (5.1)	0
	Enrofloxacin	59 (100)	0	0
	Levofloxacin	24 (40.7)	17 (28.8)	18 (30.5)
	Norfloxacin	59 (100)	0	0
Polipeptid	Bacitracin	59 (100)	0	0
Tetracyclines	Tetracycline	41 (69.5)	2 (3.4)	16 (27.1)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	45 (76.3)	1 (1.7)	13 (22)
Aminoglycosides	Gentamicin	15 (25.4)	0	44 (74.6)
Phenicols	Chloramphenicol	26 (44.1)	4 (6.8)	29 (49.2)
β-lactam/*β*-lactamase inhibitör combinations	Amoxicillin-clavulanic acid	5 (8.5)	5 (8.5)	49 (83.1)
Penicillins	Ampicillin	56 (94.9)	0	3 (5.1)
Cephems	Cefoxitin	1 (1.7)	3 (5.1)	55 (93.2)
	Cefpodoxime	9 (15.3)	0	50 (84.7)
	Cefuroxime	5 (8.5)	5 (8.5)	49 (83.1)
Monobactams	Aztreonam	1 (1.7)	1 (1.7)	57 (96.6)
Penems	Imipenem	0	0	59 (100)
Lipopeptides	Colistin	0	0	59 (100)

Table 3. Antimicrobial resistance class pattern distribution for 48 multidrug-resistant *Escherichia coli* isolates from chicken meat

Antimicrobial resistance class patterns	No. of classes	Frequency	No. of multidrug-resistant *E. coli* (%)
PCNs, PPs, FPIs	3	3	6.3
PCNs, PPs, TETs		1	2.1
PPs, FPIs, TETs		1	2.1
PCNs, PPs, TETs		1	2.1
PCNs, PPs, AMGs		1	2.1
PCNs, PPs, AMGs, FPIs	4	1	2.1
PCNs, PPs, FPIs, TETs		9	18.6
PCNs, PPs, PHs, FPIs		1	2.1
PCNS, PPs, AMGs, FPIs, TETs	5	2	4.2
PCNS, PPs, PHs, FPIs, TETs		11	22.9
PCNS, PPs, PHs, AMGs, FPIs		1	2.1
BL/BLICs, PCNs, CEPs, PPs, FPIs		1	2.1
PCNs, CEPs, PPs, PHs, FPIS, TETS	6	1	2.1
PCNs, CEPs, PPs, PHs, AMGs, FPIS		1	2.1
PCNs, PPs, PHs, AMGs, FPIS, TETS		7	14.5
PCNs, CEPs, PPs, PHs, AMGs, FPIs, TETs	7	1	2.1
PCNs, CEPs, PPs, MONs, AMGs, FPIs, TETs		1	2.1
BL/BLICs, PCNs, PPs, CEPs, AMGs, FPIs, TETs		1	2.1
BL/BLICs, PCNs, CEPs, PPs, PHs, FPIs, TETs		2	4.1
BL/BLICs, PCNs, PPs, CEPs, PHs, AMGs, FPIs, TETs	8	1	2.1
Total		**48**	**(100)**

AMGs, aminoglycosides; BL/BLICs, *β*-lactam/*β*-lactamase inhibitor combinations; CEPs, cephems; FPIs, folate pathway inhibitors; FPIS; PCNs, penicillins; MONs, monobactams; PHs, phenicols; TETs, tetracyclines; PPs, Polipeptids.



Isolate ID	CIP (MIC; µg/ml)	Resistance profiles*	Resistance genes
VGH032	≥32	AM, B, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH054	≥32	AM, B, CN, SXT, CIP, ENR, NA, NOR	TEM
VGH058	≥32	AMC, AM, B, CPD, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH057	≥32	AM, B, CIP, ENR, LEV, NA, NOR	-
VGH029	≥32	AM, CPD, CXM, B, C, CN, SXT, TE, CIP, ENR, LEV, NA, NOR	-
VGH014	≥6	AM, B, CIP, ENR, NA, NOR	-
VGH031	≥8	B, CIP, ENR, NA, NOR	-
VGH027	≥6	AM, CPD, CXM, B, C, SXT, TE, CIP, ENR, NA, NOR	CTX-M + TEM
VGH020	≥32	AM, B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH021	≥32	AM, B, CIP, ENR, NA, NOR	-
VGH034	≥32	AM, B, CIP, ENR, NA, NOR	TEM
VGH039	≥32	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH009	≥6	AM, B, SXT, CIP, ENR, NA, NOR	-
VGH046	≥4	AM, B, C, SXT, TE CIP, ENR, NA, NOR	TEM
VGH055	≥32	AM, CPD, B, C, CN, SXT, CIP, ENR, LEV, NA, NOR	TEM
VGH040	≥32	AM, B, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH048	≥32	AM, B, TE, CIP, ENR, NA, NOR	TEM
VGH023	≥32	AM, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH060	≥32	AM, B, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH013	≥>32	AM, B, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH028	≥32	AM, B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH001	≥32	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH022	≥16	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH044	≥32	AM, B, C, SXT, CIP, ENR, NA, NOR	TEM
VGH012	≥32	AM, B, CIP, ENR, NA, NOR	-
VGH042	≥8	AM, B, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH043	≥8	AM, B, C, CN, SXT, CIP, ENR, NA, NOR	TEM
VGH010	≥32	AM, B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH026	≥16	AM, B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH052	≥12	AM, B, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH033	≥8	B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH038	≥32	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH041	≥8	AMC, AM, B, FOX, CN, SXT, TE, CIP, ENR, NA, NOR	CMY-2 + TEM
VGH047	≥32	AM, B, CIP, ENR, NA, NOR	TEM
VGH007	≥32	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH011	≥4	AM, B, C, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH002	≥32	AM, B, C, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH017	≥32	AM, B, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH015	≥32	AMC, AM, CPD, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH036	≥32	AMC, AM, CPD, B, SXT, CIP, ENR, NA, NOR	TEM
VGH025	≥16	AM, B, C, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH049	≥32	AM, B, SXT, CIP, ENR, LEV, NA, NOR	-
VGH037	≥8	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH024	≥32	AM, B, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH004	≥32	AM, B, C, CN, SXT, TE, CIP, ENR, NA, NOR	TEM
VGH056	≥32	AM, B, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH003	≥32	AM, B, C, CN, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH005	≥32	AM, B, SXT, CIP, ENR, LEV, NA, NOR	-
VGH035	≥32	AM, CPD, CXM, B, ATM, CN, SXT, TE, CIP, ENR, LEV, NA, NOR	CTX-M + TEM
VGH008	≥32	AM, B, C, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH019	≥32	AMC, AM, CPD, B, C, SXT, TE, CIP, ENR, NA, NOR	CMY-2 + TEM
VGH051	≥32	AM, B, CIP, ENR, LEV, NA, NOR	-
VGH059	≥32	AM, B, SXT, TE, CIP, ENR, LEV, NA, NOR	TEM
VGH050	≥32	AM, B, C, CN, SXT, TE, CIP, ENR, LEV, NA, NOR	-
VGH053	≥8	AM, B, CN, CIP, ENR, NA, NOR	TEM
VGH018	≥32	AM, B, CIP, ENR, LEV, NA, NOR	-
VGH045	≥32	AM, B, CIP, ENR, LEV, NA, NOR	-
VGH016	≥32	AM, B, CN, SXT, TE, CIP, ENR, NA, NOR	-
VGH030	≥12	B, TE, CIP, ENR, NA, NOR	-

*AMC, amoxicillin-clavulanic acid; AM, ampicillin; ATM, aztreonam; FOX, cefoxitin; CXM, cefuroxime; CPD, cefpodoxime; C, chloramphenicol; CN, gentamicin; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin; LEV, levofloxacin; NOR, norfloxacin; SXT, trimethroprim-sulfamethoxazole; B, bacitracin. All *E. coli* strains were susceptible to imipenem and colistin, these two antimicrobials were net shown in the resistant profiles. - Not detected.

Figure 1. PFGE analysis of ciprofloxacin-resistant *Escherichia coli* isolates.

Table 4. Molecular characterization of *bla* and PMQR genes among ciprofloxacin-resistant *Escherichia coli* isolates (n = 59)

***bla* gene and PMQR**	**No. of isolates n (%)**
TEM	40 (67.8)
TEM and CTX-M	2 (3.4)
TEM and CMY-2	2 (3.4)
CTX-M and CMY-2	2 (3.4)
SHV	-
qnr A, B ,S, C and *aac (6'-lb-cr*)	-

- Not detected

DISCUSSION

The use of antimicrobials as growth promotion agents in food-producing animals has been prohibited in Turkey since 2006 (RG, 2006). Recently, Turkish Poultry Meat Producers and Breeders Association data also highlighted the decreasing trend in the use of antimicrobial drugs in poultry production in Turkey (Elmas et al., 2019). However, the occurrence of CR *E. coli* was determined as 98.3% in the current study, clearly showing a high level of contamination for chicken meat on the retail market in Turkey. The results obtained in the present study are not surprising because Ghodousi et al. (2015) reported 88.8% of CR *E. coli* isolates from chicken meat in Italy. For example, even though quinolone-based antibiotics have been not in use for poultry production, an increase in quinolone-resistant *E. coli* rates in broiler production processes were reported in Sweden (Borjesson et al., 2016). However, the results of this study were found to be much higher than the results from other countries, for example 37.4% in China (Xu et al., 2014) and 26% in the Czech Republic (Literak et al., 2013).

According to the PFGE analysis, CR *E. coli* isolates from chicken meat were clonally different. Such a difference can be attributed to the movements of humans, chickens, and vectors, and indicates that the contamination might not originate from a single source (Jakobsen et al., 2010; Sola-Gines et al., 2015). For example, in a study of *E. coli* isolated from slaughter animals in Poland, Wasyl et al. (2014) identified quinolone resistance mechanisms and noted chromosome-encoded quinolone resistance did not result from the spread of a single resistant clone, rather this was due to antimicrobial pressure leading to the selection of random *gyr* and *par* mutants.

In the present study, CR *E. coli* isolates from chicken meat samples were extremely resistant to AM (100%), SXT (76.3%), and TE (69.5%). For instance, in a previous study conducted by Ghoudousi et al. (2015) in Italy, 134 *E. coli* were isolated from 109 chicken meat samples, and their resistance patterns were found for CIP (88.8%), CN (14.1%), SXT (79.1%), and TE (91.8%). In a study conducted by Soufi et al. (2011), 166 *E. coli* isolates were obtained from poultry meat (whole carcasses of chickens and turkeys) in Tunisia, and a significantly high percentage of resistance to ampicillin, nalidixic acid, sulfonamides, and tetracycline (66-95%) were observed among the isolates from poultry meat. Moreover, in another study performed by Xu et al. (2014) in China, all of the *E. coli* isolates were also resistant to AM (100%), SXT (94.3%), and TE (94.3%). High rates of resistance were observed for tetracycline (69.5%) in this study and were similar to the results reported from other countries (Soufi et al., 2011; Ghodousi et al., 2015; Xu et al. 2014). This finding is not surprising, as quinolone-resistant *E. coli* isolates were found from broilers in Sweden, which never approved these antimicrobials in the poultry industry.

In the current study, the CR *E. coli* isolates were mostly resistant to quinolone antibiotics, except for LEV. This resistance could be related to the levofloxacin molecule's C-8 methodxy group and the fluorinated quinoloic acid cores, which is different than the ciprofloxacin (Fu et al., 2013; Lu et al., 2001).

In this study, data showed that all 59 CR *E. coli* isolates had high levels of phenotypical MDR profiles (81.4%; n = 48) for the chicken meat in Turkey, which were resistant to three to eight classes of antimicrobial agents. Similar studies from the Czech Republic, Italy, and China found MDR *E. coli* in retail chicken meat at 82%, 66.9% and 59.4%, respectively (Ghodousi et al., 2015; Literak et al., 2013; Xu et al., 2014). In a study performed by Seo and Lee (2019) in Korea with 248 chicken meat samples, 152 isolates were observed to be positive for *E.coli*; 75 were identified as MDR *E. coli.* Of the MDR *E. coli* isolates, 13.3% were observed to be positive for PMQR genes, and 41.3% of the MDR *E. coli* isolates were found to be carrying class 1 integrons. The authors suggested

that PMQR genes and class 1 integrons were widely distributed in *E. coli* isolates from chicken meat and were contributed to resistance to diverse antimicrobial agents. In contrast, no PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD*, and *aac(6')-Ib-cr)* were detected in this study. The PMQR genes were rare for Turkey (Kürekci et al., 2018; Müştak et al., 2012). However, CR *E. coli* was found with high rates in this study, and the occurrence of PMQR determinants was not always related with quinolone resistance. Yang et al. (2014) reported an increased prevalence of PMQR traits from 6.2–28.1% in 2004/2005 to 23.2–50.4% in 2010/2011, while ciprofloxacin resistance was relatively stable during the study period in China.

Several studies have shown the prevalence of PMQR genes in *E. coli* isolates from chicken meat products (Ghodousi et al., 2015; Literak et al., 2013; Yu et al., 2015). In this study, none of the PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD*, and *aac(6')-Ib-cr)* were determined to be present in the 59 *E. coli* isolates. The prevalence of the PMQR genes (*qnrA*) in Italy was determined as 91% in nearly all of the *E. coli* isolates from the chicken meat samples (Ghodousi et al., 2015). On the other hand, PMQR genes were reported as 4% and 7.8% in the Czech Republic and China, respectively (Literak et al., 2013; Yu et al., 2015). Additionally, in other studies carried out in Turkey, a low-level presence of the PMQR gene was reported (Kürekci et al., 2018; Müştak et al., 2012). Previously in Turkey, Müştak et al. (2012) reported the presence of only the *qnrA* of the PMQR gene in five (5.3%) samples of 94 isolated chicken *E. coli* cloacal swaps. More recently, *qnrS* (n = 5) and *qnrB* (n = 8) of the PMQR genes from chicken meat were reported among the ESBL-producing *E. coli* strains obtained from chicken meat samples; however, *qnrA, qnrC, qnrD*, and *aac(6')-Ib-cr* genes were not found (Kürekci et al., 2018).

Quinolones are known to occur mainly through the accumulation of target enzyme point mutations. DNA gyrases (*gyrA* and *gyrB*) tend to be the primary target of quinolone in gram-negative bacteria, including *E. coli,* whereas in gram-positive bacteria, topoisomerase IV (*parC* and *parE*) is the primary target (Vanni et al., 2014). However, in the current study, *E. coli* isolates were not examined for chromosomal mutations. In addition, only PMQR genes were investigated in *E. coli* isolates, and PMQR genes were found in none of the isolates. In a study conducted by Xu et al. (2014), four topoisomerase point mutations showed ciprofloxacin MIC ≥ 32 μg/mL in all *E. coli* isolates, and the authors noted that higher ciprofloxacin MIC usually had more complex quinolone-resistant determinants, including PMQR mechanisms. In the same study (Xu et al., 2014), PMQR determinants were identified in more than 60% of the *E. coli* isolates. Xu et al. (2014) also noted the MIC value of ciprofloxacin was highly related to the accumulation of a resistance mechanism.

A meaningful correlation has been reported between ESBL (together with CTX-M types) and PMQR genes (Branger et al., 2005; Nordmann and Poirel, 2005). In a recent study of ESBL *E. coli* in milk and chicken meat samples by Kürekci et al. (2018), a significantly high rate of ESBL (86.7%) *E. coli* strains were reported to be isolated, and the authors noted that the CTX-M gene was quite prevalent (62.3%) in these isolates. In this study, 8.3% of the isolated CR *E. coli* was determined to be ESBL-positive, and two isolates (3.4%) possessed the $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ genes, whereas 40 isolates (67.8%) possessed the bla_{TEM} gene. Similar findings were reported from other studies (Cohen Stuart et al., 2012; Xu et al., 2014; Yu et al., 2015). Moreover, all ampicillin-resistant isolates were positive for the presence of the bla_{TEM} gene. Literak et al. (2013) reported similar results for broiler samples in the Czech Republic.

CONCLUSION

The current study showed high rates of CR *E. coli* among retail chicken meat samples. Therefore, the prudent use of antimicrobial agents is an important issue to poultry farmers in Turkey. Although MDR was found to be high, the absence of the PMQR gene was favorable in terms of a public health concern. Hence, continuously investigating the MDR level and the feasibility of the limitations of antibiotics and the restrictions is essential.

CONFLICT OF INTEREST

None declared by the authors.

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